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THE ACTION OF DFP ON NEURO MUSCULAR TRANSMISSION IN THE RAT

By

R. HOLMES

PORTON TECHNICAL PAPER No. 482

C.D.E.E.
Porton.
Wilts.

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Porton Technical Paper No. 482
Date: 18 APR 1955

THE ACTION OF DFP ON NEURO-MUSCULAR TRANSMISSION IN THE RAT

By

R. Holmes

SUMMARY

1. In rats protected from asphyxia by artificial ventilation and atropine, it has been shown that DFP causes an increase in muscle conduction velocity and a decrease in neuromuscular transmission time.

2. The possible mechanism of this is discussed.

(Sgd.) C. Lovatt Evans,
Head, Physiology Section.

(Sgd.) H. Cullumbine,
Supt., Medical Division.

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recorded, this one originating at the distal end-plate zone and appearing inverted since it crosses the recording electrodes in the reverse direction to the first spike. This was described by Berman et al, and is again illustrated in figure 1.C. In order to obtain an indication of changes at the neuromuscular junction, these authors measured the time between the stimulus artifact and the first spike, this being referred to as transmission time and including three intervals: that for nerve transmission, end-plate delay, and muscle conduction to the recording electrodes. The same interval has been used in the present work.

Muscle conduction velocity was estimated by Berman et al by recording with two pairs of electrodes, one near to each end-plate zone. The time taken for a spike to travel from one to the other pair of electrodes was obtained and a measurement of interelectrode distance enabled the conduction velocity to be obtained. In the present work, this was not done. To obtain a measure of conduction velocity changes, the interval between the two spikes recorded by one pair of electrodes was measured. If the electrodes are placed very close to an end-plate zone, this time interval will approximate closely to the time taken for the muscle action potential to travel between the two end-plate zones. The interval will deviate from this owing first to the additional nerve conduction time to the distal end-plate zone and secondly to the interval between the recording electrodes and the proximal end-plate zone.

In the majority of the experiments no measurements of interzonal distance have been made, variations in time interval alone being taken. In other experiments however, the rat was fully curarized after the records had been taken and the interzonal distance determined by measuring between the maxima of the end-plate potentials.

Berman et al found that respiratory failure occurred early in DFP poisoned rats and could not be overcome by artificial positive pressure ventilation, although this delayed the cardio-vascular collapse for a short time. Various attempts were made to overcome this, for example both vagi were cut before poisoning to avoid potentiation of any tonic cholinergic discharges to the heart and lungs. Also the chest was opened widely so that the positive pressure ventilation would not impede the venous return. These manoeuvres were however, unsuccessful, the heart still ultimately failing. In order therefore to prevent this and avoid the recording of events in a dead animal, the rats were atropinized at a dose level of 1 mg/Kg. given intravenously some 15 minutes before injection of the DFP. 2.5 mg/Kg. of DFP i.v. was used as the standard dose, this being a little over 1.5 L.D. 50's and therefore almost certainly lethal. The animals were artificially ventilated throughout the experiments by means of a Palmer small-animal pump used at a rate of 100 per minute with a volume of 6 cc. The nerve to gracilis was stimulated with single shocks from a square-wave stimulator at a rate of 10 per minute and the voltage was adjusted to supra maximal.

In some experiments the muscle was stimulated directly. To avoid stimulation of the muscle through fine nerve trunks the stimulating electrodes were placed as close to the pelvic attachment as possible, and the stimulus strength adjusted so that it was just a little above threshold for only a small number of fibres.

The electrocardiogram was recorded so that any changes could be observed, and the heart rate was counted from the loud-speaker connected with the E.C.G. amplifier.

Action potentials and time were photographed on 35 mm. film, enlarged about 10 times and the various intervals measured to the nearest mm. 1 m.sec. was usually equivalent to 24 mm.

Results

It has been found consistently in each of the six experiments of this group, that DFP causes an increase in the muscle conduction velocity as indicated by a decrease in the interval between the two muscle spikes. The results are shown in table 1.

TABLE 1

Interval between spikes (m.sec)

Rat	Control	Minimum after DFP	Difference (m.sec)	% Difference
1	2.70	2.08	0.64	23.7
2	2.82	2.25	0.57	20.2
3	2.42	1.97	0.45	18.6
4	2.62	2.01	0.61	23.3
5	3.05	2.58	0.47	15.4
6	2.52	2.12	0.42	16.7
Means	2.69	2.16	0.53	19.6

With a conduction distance of 11 mm., conduction velocity would increase from 4.1 to 5.1 m/sec.

Muscular fasciculations occurred about 1 minute after the injection of DFP and continued throughout the experiment (up to 1 hour after injection). The decrease in conduction time did not however occur with similar rapidity but developed slowly, reaching a minimum between 10 and 20 minutes after injection. This is shown in figure 1a. Owing to the protection afforded by the atropine, the heart rate changed little throughout the experiment and the E.C.G. remained normal.

To confirm that an increase in muscle conduction velocity occurred after DFP poisoning, the muscle was stimulated directly and muscle action potentials recorded some 2 or 3 cms. distant. Here also, an increase was found as shown in figure 2. The distance between stimulating and recording electrodes was 28 mm. the conduction velocity therefore increasing from 4.23 to 4.70 m/sec.

Berman et al found that conduction velocity was slowed after tracheal occlusion and this has been confirmed in the present work. The changes are small however (e.g. fig.3a) since recording was not continued after the ventricles had ceased to beat, (between 6 and 10 minutes after clamping the trachea). Berman et al continued to record in 2 of 4 experiments for 25 minutes. In addition to confirming the slowing of muscle conduction a further group of three experiments were done to determine whether atropine caused any difference from this result. Previous control values showed that there was no change on injecting atropine 1 mg/Kg. and it was also found that atropine did not alter the response to tracheal occlusion. Again a slight increase (0.16 m.sec.) in conduction time was obtained. The decrease in conduction time in the DFP poisoned animals can therefore not be attributed to the atropine.

Measurement of the interval between the stimulus artifact and the first spike shows that the "transmission time" also decreases in rats poisoned with DFP. The reduction was generally less than that found for conduction times. When expressed as a percentage difference, it is seen that there is a wide scatter of the values which does not appear to be correlated with the control value. The values are given in Table 2 and fig.1.D., and shows the time course of the change.

TABLE 2

Transmission Time (m.sec.)

Rat	Control	Minimum after DFP	Differences (m.sec.)	% Difference
1	1.02	0.89	0.13	23.7
2	1.84	1.64	0.20	20.2
3	2.31	1.86	0.45	18.6
4	1.56	1.17	0.39	23.3
5	1.36	0.98	0.39	15.4
6	2.30	2.13	0.17	16.7
Means	1.73	1.45	0.28	15.6

From a purely qualitative view the results do however show that DFP administration causes a reduction in transmission time when the rat is protected from asphyxia.

Tracheal occlusion was found to cause little change in transmission time (fig.3b) up to the time of cessation of ventricular contractions as indicated by the QRS complex of the ECG. This agrees with the findings of Berman et al. The same absence of any change after tracheal occlusion was also shown in atropinized animals.

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THE ACTION OF DFP ON NEURO MUSCULAR TRANSMISSION IN THE RAT

ERRATUM

Page 4, Table 2. Delete figures in column headed "% Difference" and replace by

12.7
10.9
19.5
25.0
28.7
7.4
17.4

PORTON
4th May, 1955.
CEL/GC.

Discussion

Berman et al found that DFP poisoning in the rat resulted in a slowing of muscle conduction velocity which closely resembled that produced by asphyxia. Since an opposite result has been obtained when asphyxia was prevented by administration of atropine, it is suggested that the slowing found by Berman et al was due to asphyxia and was related only indirectly to the DFP poisoning. They did however note an initial stimulatory effect on muscle conduction velocity before this was slowed by asphyxia, and this stimulation has been consistently observed in the present work, both to indirectly and directly applied electrical excitation. The increase in conduction velocity was maintained for periods of up to 60 minutes. Transmission time was decreased, this effect also being maintained.

Although much work has been done on the effects of DFP on neuromuscular transmission, it has mostly been concerned either with the recording of whole muscle contractions, or with changes in the end-plate potential. The present work suggests an additional effect in causing an increase in muscle conduction velocity. The mechanism by which it occurs is however not necessarily due to a a direct action of the DFP on the muscle fibre. That the DFP was acting at the motor end-plates was shown by the response to tetanic stimulation of the nerve when the muscle action potentials become successively smaller (figure 4), demonstrating typical Wedeasky block. Single shock stimulation of the nerve therefore caused a prolonged end-plate potential which as shown by Fatt and Katz (1951) would involve depolarization of the adjacent muscle membrane. The result of this would be a reduction in the effective length of conducting muscle between the two end-plate zones. That a decrease in conduction time was also found when the muscle was stimulated directly would appear to counter this argument, but it should be noted that the stimulus was applied close to the pelvic attachment of the muscle and the recording electrodes placed at about the centre of the muscle. The muscle spikes would therefore have to traverse an end-plate zone. Burns and Paton (1951) have shown that conduction of a muscle spike can be blocked if the end-plate is fully depolarized (by decamethonium in this case). It is one of the characteristics of depolarizing drugs that block is preceeded by excitation so that if the end-plate were partially depolarized (by circulating acetyl choline, perhaps) the muscle conduction would be facilitated at this region. These considerations would apply to the present experiments where the end-plate zone is in a state of facilitation as a result of DFP injection as shown by repetitive discharges in the muscle fibres.

SUMMARY

1. In rats protected from asphyxia by artificial ventilation and atropine, it has been shown that DFP causes an increase in muscle conduction velocity and a decrease in neuromuscular transmission time.

2. The possible mechanism of this is discussed.

(Sgd.) C. Lovatt Evans,
Head, Physiology Section.

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Supt., Medical Division.

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Jarcho L.W., Eyzaguirre C., Talbot S.A. and Libenthal J.L.	(1950)	Amer J. Physiol 162 475

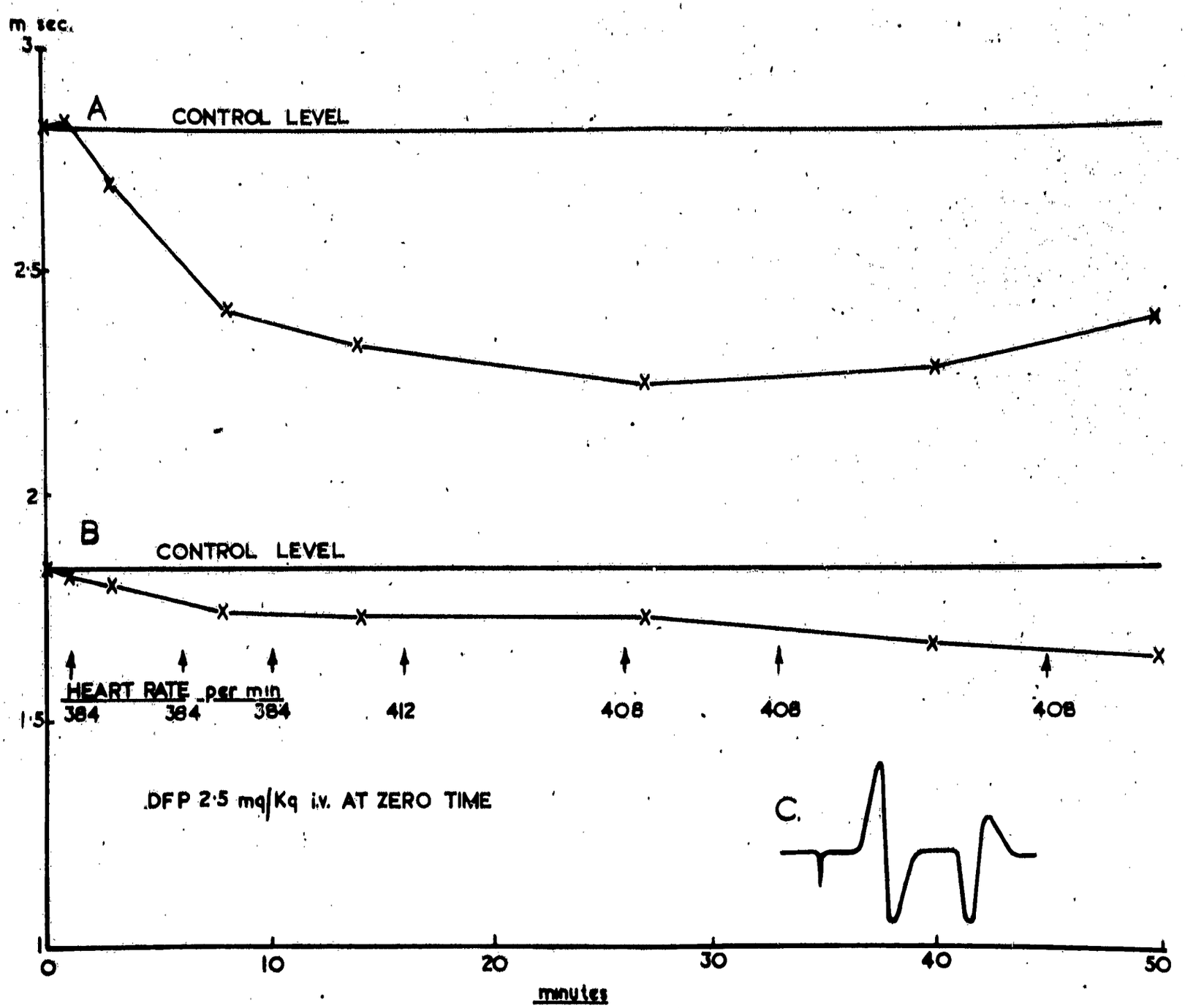
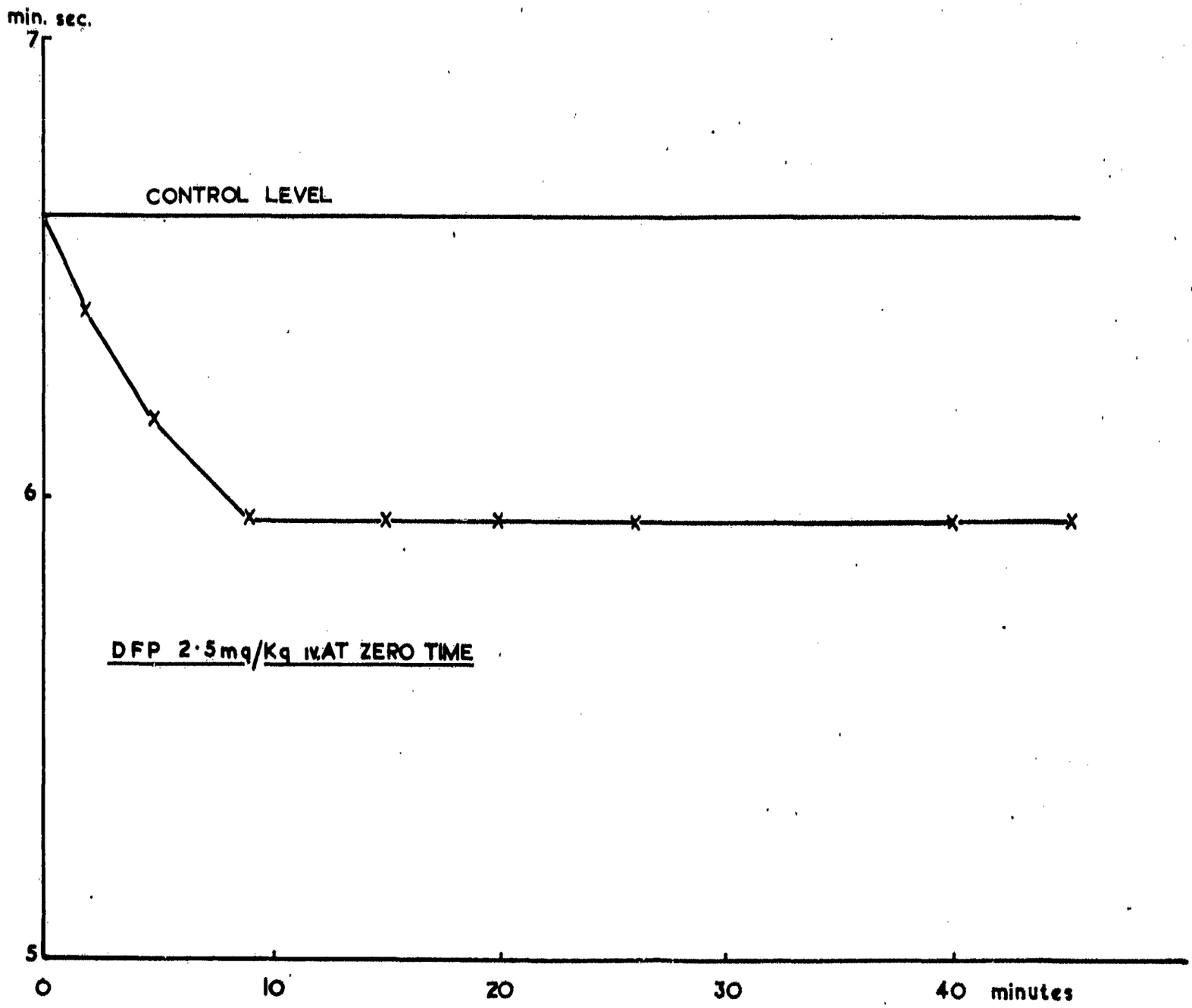

m sec.
3
2·5
2
1·5
1
A
CONTROL LEVEL
B
CONTROL LEVEL
HEART RATE per min
384
384
384
412
408
408
408
DFP 2·5 mg/Kg i.v. AT ZERO TIME
C.
0
10
20
30
40
50
minutes

FIG. 1


min. sec.
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CONTROL LEVEL
6
DFP 2·5mg/Kg IV. AT ZERO TIME
5
0
10
20
30
40 minutes

FIG. 2

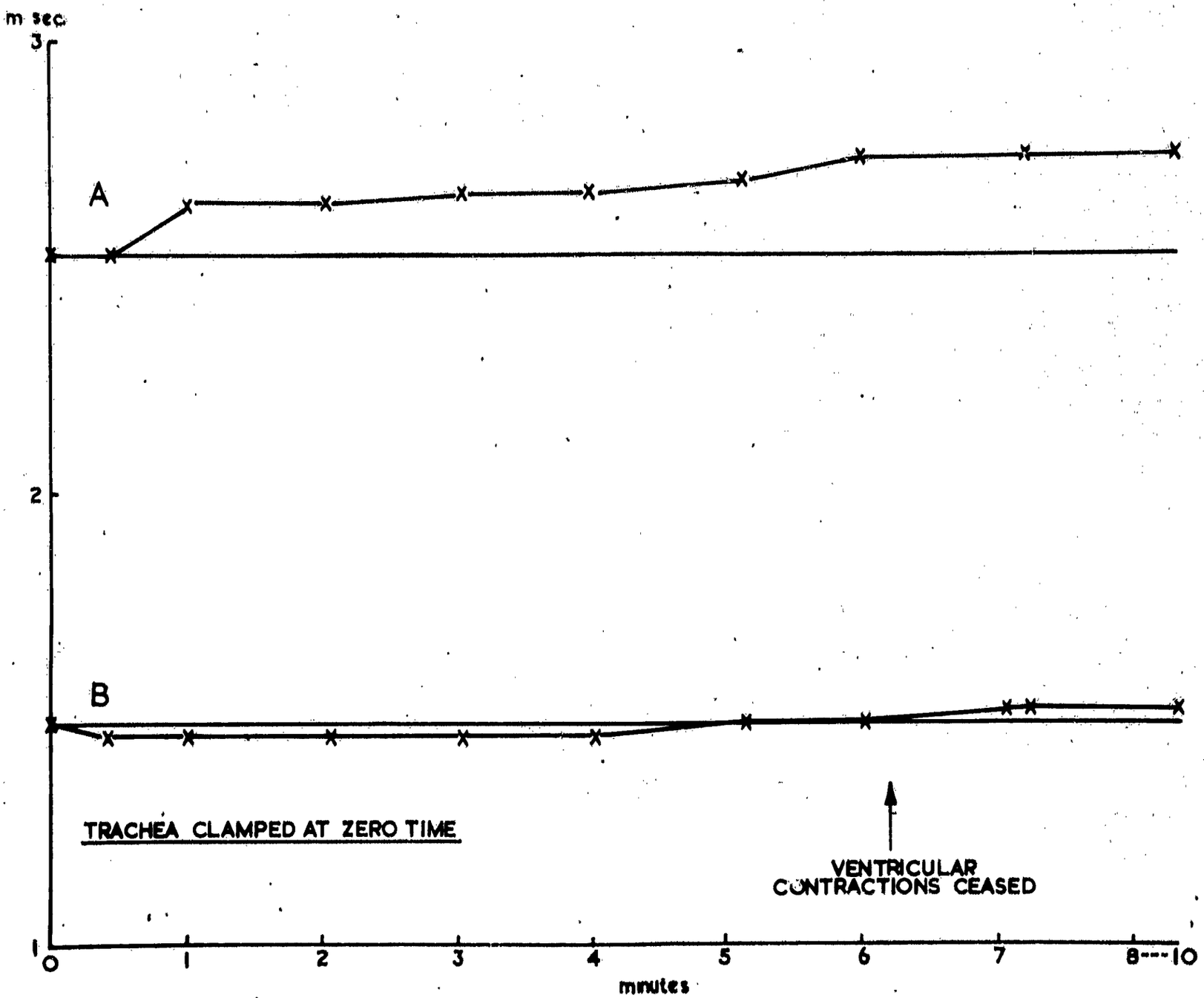
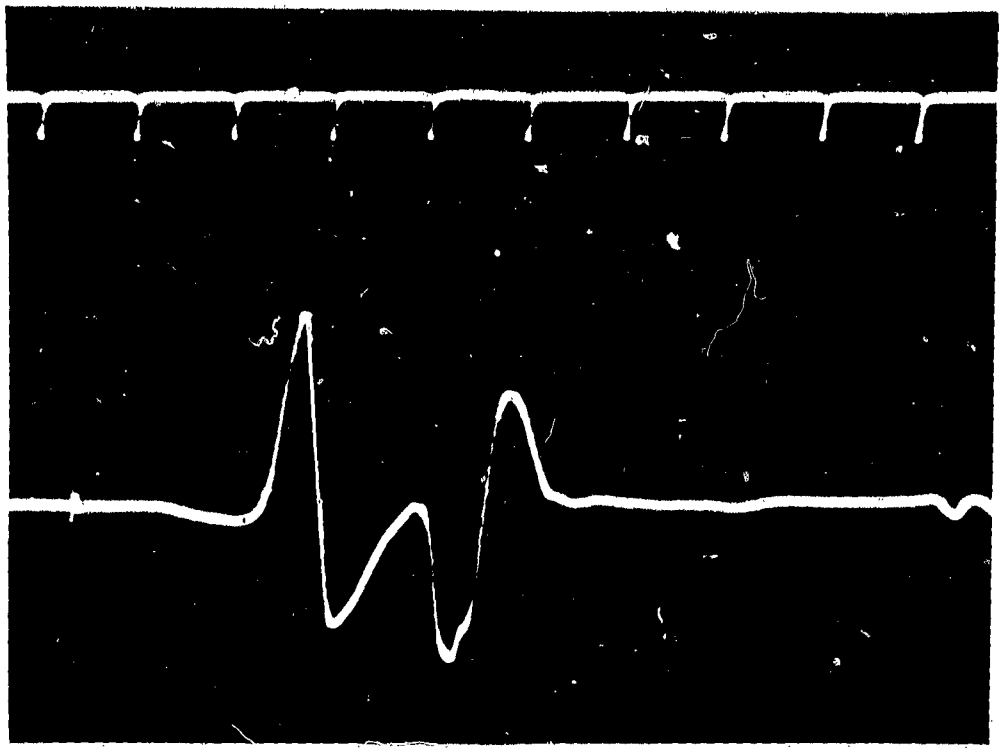

m sec.
3
2
1
A
B
TRACHEA CLAMPED AT ZERO TIME
VENTRICULAR
CONTRACTIONS CEASED
0
1
2
3
4
5
6
7
8---10
minutes

FIG 3

Fig 4



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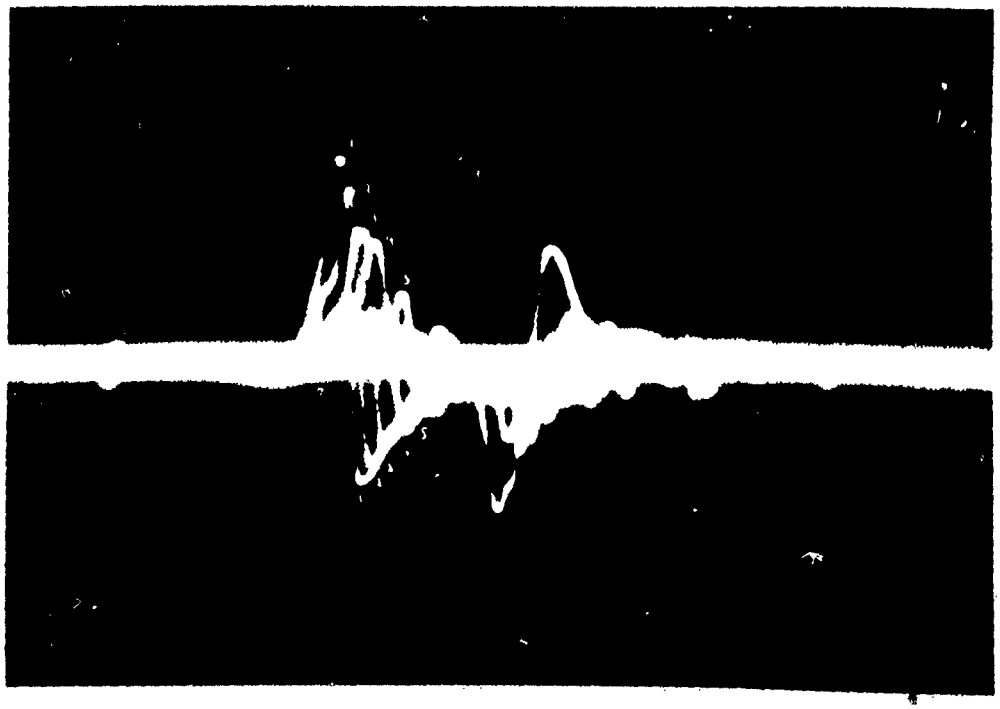

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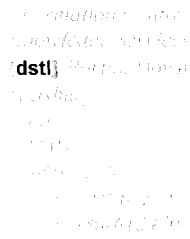

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